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Phosphorus is routinely applied to soils in tree-based restoration schemes, despite the balance between beneficial and deleterious effects being poorly understood. For six woody species from the Great Western Woodlands of Western Australia, growth increased in response to low concentrations of applied phosphorus. At higher phosphorus concentration, growth either plateaued or declined. Applied phosphorus may have different and unpredictable effects on native species suggesting caution when applying high rates of phosphorus in a field-setting.

Sensitivity of seedling growth to phosphorus supply in six tree species of the Australian Great Western Woodlands

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Abstract

Many Australian native plants from regions with ancient, highly weathered soils have specialised adaptations for acquiring phosphorus (P) and can exhibit negative effects of excess P supply on growth and survival. Despite this, fertiliser (including P) is routinely applied in post-mining and other restoration schemes. In this study we investigated the effect of a range of applied P on the growth and tissue P concentrations for six woody species from the Great Western Woodlands (GWW) of Western Australia, a region that it not only biodiverse, but that has experienced significant levels of mining related activities. Our data from a pot-based experiment show that all six species exhibited greater growth with increased P application up to 15 mg kg sand^{-1}. However, at P concentrations in excess of 15 mg kg^{-1}, dry mass accumulation did not increase further for three of the species tested. For the other three species, dry mass accumulation declined as the P concentration increased above 15 mg kg^{-1}. For all of the study species, root and shoot P concentrations increased as the concentration of applied P increased. The internal shoot P concentration, at which dry matter accumulation either plateaued or started to decline, was in the range 1.95 to 3.2 mg P g^{-1} DM. This was approximately 2 to 4 times the concentration found in natural vegetation. These data suggest that in a restoration context, there is a potential risk that, excess P applcation may decrease plant growth rates for some species. Consequently, the addition of fertiliser to restored sites may have unpredictable impacts on the plant community by directly reducing the growth of some species while increasing the growth of others. We suggest that careful consideration should be given to designing appropriate fertiliser regimes for land restoration schemes in ancient P deplete landscapes to avoid the risk that fertiliser addition has the unwanted outcome of decreasing growth and survival of the target native species whilst increasing the abundance of unwanted weeds or aggressive pioneer species.

Key words: fertiliser, P accumulation, P-toxicity, P-use efficiency, rehabilitation

Introduction

The Great Western Woodlands (GWW) of Western Australia is the largest intact temperate woodland left on earth (Morton 2014). The region hosts a plethora of biodiversity including 3,300 flowering plants, approximately 30 % of Australia's *Eucalyptus* species and 138 reptile species (Booth 2013). Whilst being a biodiversity hotspot, the GWW also contains rich mineral resources and has been subjected to mining: the GWW have significant geographical overlap with the Western Australian goldfields (Watson et al. 2015). As a result, there is a need for a greater understanding of the growth, physiology and nutrition of native tree species for use in post-mining restoration.

The addition of fertiliser to increase plant growth in newly established post-mining restoration is a common feature of mine restoration programmes both in Australia and elsewhere (e.g. George et al. 2006; Worrall et al. 2007; Standish et al. 2010; Tibbett 2010; Williamson *et al*. 2011; Zipper *et al*. 2011; Ortiz *et al*. 2012; Spain *et al*. 2019). However, an increasing number of studies suggest that there may be contrasting effects of fertiliser addition on different functional groups of species. For example, addition of phosphorus (henceforth, P-fertiliser) to mine restoration in the Cape Region of South Africa and in SW Western Australia increased the growth of both native and non-native annual species, but either increased mortality or reduced the establishment of slower growing species such as Proteaceae (Holmes 2001; Daws *et al*. 2013, 2015).

The Cape Floristic Region and SW Western Australia have soils depleted in nutrients, particularly P, due to a preponderance of ancient, highly weathered soils (Handreck 1997; Lambers *et al*. 2008;). Consequently, many plant species in these environments have specialised adaptations, such as root clusters, mycorrhizal symbioses and exudation of carboxylases and phosphatases, for acquiring P (Lambers *et al*. 2006, 2008). Furthermore, a number of Australian plant species occurring on nutrient impoverished soils have a limited ability to regulate P-uptake when the P-supply is increased. These species are sensitive to P-toxicity when supplied with P concentrations above those that they naturally experience in soil (Shane *et al*. 2004a; Handreck 1991; Lambers *et al*. 2002; Pang *et al*. 2010; de Campos *et al*. 2013), due potentially to the loss of low affinity transporter systems (Huang *et al*. 2011). Consequently, when undertaking environmental restoration in environments with naturally nutrient deficient soils, there may not just be little benefit to growth from adding (P-) fertiliser, but also potential negative effects on growth and survival for a range of species. However, many of these studies on responses to applied-P have either focused on Proteaceae or on species from the naturally P-deficient soils of SW Western Australia (or both). As a result, there is a need to investigate plant responses to applied-P in other nutrient deficient regions.

The soils of the GWW are also typically deficient in P and consequently, the application of fertiliser, particularly P, to increase plant growth is viewed as a requirement to increase growth and establishment in post-mining restoration in this region (e.g. KCGM 2015). However, there is minimal available data of the effects of P-addition, on species from the GWW, particularly with regard to potential problems such as P-toxicity.

Consequently, our current study was established to determine how tree seedlings native to the Australian GWW respond to a wide range of P concentrations. For six species we tested, (1) their response to low-P (P-response efficiency), (2) their capacity to grow and regulate P-uptake at high-P, where toxicity might depress growth, and (3) the response of root and shoot tissue P concentrations to applied P.

Materials and methods

Plant material and experimental design

Six tree and shrub species from three genera, all native to the GWW of Western Australia, were used in the current study (see Table 1). All six species are used in post-mining restoration in this region. This study was carried out in a shade house established in Kalgoorlie in the South Western Interzone in the Coolgardie Botanical district in WA, Australia (30°44′56′′ S, 121°27′57′′ E; Beard 1990) for the study. All seeds used were subjected to appropriate pre-treatments (e.g. application of smoke water) to enhance germination, following which the seeds were planted into a sand substrate.

Seeds were sown in October 2006 with seedling growth occurring from November 2006 to April 2007 for a total period of 136 days. This time of year was chosen for the experiment as it is the optimal time for growth in this region. Temperatures during this period ranged from 13.6 to 36.9 °C. Temperature in the shade house was not regulated and reflect the range of temperatures typical for the GWW area.

For each of the six species, four replicate pots containing 5 kg of yellow river sand were established at each of seven phosphorus concentrations (0, 4, 8, 15, 30, 90, 150 mg P kg^{-1} sand). The sand contained 1 mg kg^{-1} Colwell P and N was below detection limits. P was added in the treatments as KH_2PO_4. Additional nutrients were provided in two aliquots throughout the experiment to give a nutrient concentration of: (mg kg^{-1} soil) K 62.8, S 39.4, Ca 40.9, Mg 7.9, Cu 0.53, Zn 2.86, Mn 3.29, B 0.12, Co 0.09, Na 0.04, Mo 0.08 and N 33.N was added in the form of NH_4NO_3 and sulphur in $MgSO_47H_2O$. Soil moisture was maintained throughout the experiment by adding deionised water to match weight loss. Plants were checked regularly for pests and signs of infection.

Plant measurements

At the end of the growth period (136 days after the start of the experiment), plants were carefully removed from the growing medium. Roots were washed with water; the plants were then separated into roots and shoots. Morphological features were noted and then samples were then oven dried at 70ºC to a constant weight. Sub samples were digested using nitric perchloric acid (1:3) and P was measured using the heteropoly-molybdenum method (Ames 1966).

For each species, growth responses (total plant dry mass) to applied-P were calculated at P supplies of 4, 8, 15, 30, 90 and 150 mg P kg^{-1}. Following the methodology in Pang *et al*. (2010), these growth responses were calculated for the following ranges of P application: 1) subtracting total dry mass for the 0 mg P kg^{-1} treatment from dry mass at 4 mg P kg^{-1}; 2) subtracting dry mass at 4 mg P kg^{-1} treatment from dry mass at 8 mg P kg^{-1}; 3) subtracting dry mass at 8 mg P kg^{-1} treatment from dry mass at 15 mg P kg^{-1}; 4) subtracting dry mass at 15 mg P kg^{-1} treatment from dry mass at 30 mg P kg^{-1}; 5) subtracting dry mass at 30 mg P kg^{-1} from dry mass at 90 mg P kg^{-1}, and 6) subtracting dry mass at 90 mg P kg^{-1} from dry mass at 150 mg P kg^{-1}. The increments in plant dry mass were then divided by the difference between amounts of P supplied to provide P-response efficiency values (dry mass produced per unit of applied P in each of the six ranges of P application, such that for each incremental increase in applied P:

$$\text{P response efficiency} = \Delta \text{ P in dry mass}/\Delta \text{ applied soil P}.$$

Statistical analysis

This investigation followed a two factor (concentration of applied-P and species) randomised block design. Two-way ANOVA implemented in Minitab 14 was used to assess, for each species, whether there were effects of increasing external P concentration on either tissue specific (root versus shoot) dry mass or P concentration. Data did not require transformation prior to analyses as the assumptions of ANOVA with respect to normality and homogeneity of variances were met.

Results

Plant biomass

Plant growth in the absence of applied-P varied between species with total dry mass (root plus shoot) ranging from 0.14 to 1.42 g for *Acacia acuminata* and *Eucalyptus torquata*, respectively (Fig. 1). Across the six species there was no correlation between seed mass and maximum plant dry mass in the absence of applied-P ($P > 0.05$). Root biomass of *Atriplex nummularia*, *Atriplex vesicaria* and *Maireana triptera* increased significantly as external P increased from 0 to 15 mg kg^{-1} and then exhibited no further change as P increased further up to 150 mg kg^{-1} (Fig. 1 c,d,f). For *Acacia accuminata*, *Acacia hemiteles* and *Eucalyptus torquata* there was little change in root biomass as soil P concentration increased (Fig. 1 a,b,e).

For all six species, shoot biomass increased as the applied P concentration increased from 0 to 15 mg kg^{-1} (Fig. 1). However, at P concentrations greater than 15 mg kg^{-1} there was either no further increase in shoot biomass (*Atriplex nummularia*, *Atriplex vesicaria* and *Acacia acuminata*; Fig. 1a,c,d) or biomass declined with increasing P (*Maireana triptera*, *Eucalyptus torquata* and *Acacia hemiteles*; Fig. 1b,e,f). For each of the six individual species the effect of increasing external P on overall dry mass was significant (Two-way Anova, $P <$ 0.05). In addition for all six species shoot biomass was significantly higher than root biomass (Two-way Anova, $P < 0.05$).

Tissue P concentration

For all six species there was a significant effect of increasing concentrations of external P on root and shoot P concentrations (Two-way Anova, $P < 0.001$; Fig. 2), with little exogenous P was required to give foliar P concentration considerably above level found in the natural flora. For *Acacia acuminata*, *Acacia hemitelata* and *Eucalyptus torquata*, tissue P concentrations exhibited little response to P concentration in the growing medium until the P concentration was approximately 15 mg kg^{-1}. Beyond this external concentration, both root and shoot P concentrations continued to increase with increasing applied-P (Fig. 2a,b,e). In contrast, for *Atriplex nummularia*, *Atriplex vesicaria* and *Maireana triptera*, tissue P concentrations increased in response to the lowest level of applied P (4 mg kg^{-1}). There were also significant differences in P concentrations between root and shoot tissues (Two-way Anova, $P \leq 0.008$). The interaction between tissue type and external P-concentration was significant (Two-way Anova, $P \leq 0.001$) for all species, except *Atriplex nummularia*, indicating differences in the response of root and shoot P concentration to increasing external P. For two of the species (*Atriplex nummularia* and *Atriplex vesicaria*), P concentrations were significantly higher in shoot than root tissue (Fig. 2a,d). For *Acacia acuminata*, root P accumulation was significantly higher than that of its shoot P, while limited significant differences in root and shoot P were observed for *Acacia hemiteles*, *Eucalyptus torquata* and *Maireana triptera*. At maximum total dry mass (approximate external P concentration of 15 mg kg^{-1}), shoot P concentrations ranged from 1.95 (*Atriplex nummularia*) to 3.72 mg g^{-1} DM (*Acacia acuminata*). For *Acacia hemitelata*, *Eucalyptus torquata* and *Maireana triptera* a decline in biomass with increasing applied P coincided with shoot P-concentrations in excess of 2.47, 2.1 and 3.2 mg g^{-1} DM, respectively.

P-response efficiency

P-response efficiency differed among the 6 species. *Atriplex nummularia* and *Eucalyptus torquata* showed the highest apparent P-response efficiency as P increased from 0 to 4 mg kg^{-1} sand while *Acacia hemiteles* showed the lowest apparent P-response efficiency (Fig. 3). When applied P increased from 4 to 8 mg kg^{-1}, *Atriplex nummularia* still had the highest P-response efficiency, but the order for the other species changed: *Maireana triptera* now had the second highest P-response efficiency whilst *Eucalyptus torquata* was ranked fourth of the six species. When applied P increased further, P-response efficiency declined in all species and was either close to zero or negative at the highest P concentrations (Figure 3).

Discussion

An increase in external P-supply resulted in an increased biomass for our study species only when the P supply was relatively low. At higher P supply, there was either no further increase in biomass or biomass declined. Similar patterns have been observed previously for a range of Australian species from severely nutrient-impoverished environments (Grundon 1972; Groves and Keraitis 1976; Handreck 1997; Pang *et al*. 2010; de Campos *et al*. 2013).

Maximum dry mass of our study species was generally observed at an external P concentration of 15 mg kg^{-1} sand. In addition, for all the study species P-response efficiency values were positive at low P-application rates (≤15 mg P kg^{-1}) indicating a continuing growth response to increasing P application. However, above 15 mg P kg^{-1}, values were either close to zero or negative suggesting that there is no further incremental benefit to growth from applying P at rates greater than 15 mg kg^{-1}. Similarly, Ryan *et al*. (2009) recorded maximum root and shoot dry mass for the Australian native *Ptilotus polystachys* at 15 mg P kg^{-1} soil. Pang *et al.* (2010), also observed maximum dry mass for four perennial legumes at an external P concentration of 12 mg kg^{-1} soil and for their remaining 7 species, maximum dry mass was achieved at 24 mg kg^{-1} soil. In addition, Pang *et al*. (2010) reported that for 8 of their study species, when the external P concentration was greater than that required to obtain maximum dry mass, total dry mass was reduced, even though most of the species did not exhibit obvious visual symptoms of P-toxicity.

Significantly higher P-concentrations were observed in roots than shoots of *Acacia acuminata*. This may reflect an ability to allocate and store excess P within the root tissue. Such a mechanism has been shown in several species of Proteaceae (Jeschke and Pate 1995; Shane *et al*. 2004b; Shane and Lambers 2006) and may serve to buffer the leaves and shoots from the effects of excess P as well as enabling P remobilisation for subsequent growth thereby contributing to highly efficient P use.

For the three species that exhibited a negative effect of elevated levels of external-P on biomass accumulation, the shoot P concentrations at which these negative effects occurred were at the lower end of the range of leaf and shoot P concentrations for P-toxicity compiled previously (0.9 to 47 mg P g^{-1} dry mass; Shane *et al*. 2004b). However, the range of values for shoot P at which negative effects were observed (2.1 to 3.2 mg g^{-1} DM) are in close agreement with those reported for P-toxicity in *Eucalyptus marginata* (1.8 to 5.5mg P g^{-1} DM) by Kariman *et al*. (2014). One possible explanation for the difference between our current values and those reported in Shane *et al*. (2004b) is that many of the values reported by Shane and co-workers are for the onset of *visible* symptoms of P-toxicity (e.g. necrosis): our results indicate the onset of a negative effect on plant growth.

It is also interesting to note that the values of shoot P that we report at the lowest external P application rates were consistent with concentrations recorded for plants in the same families harvested from the natural environment (Foulds 1993). In fact, over a wide range of habitats, Foulds (1993) only very occasionally found tissue P concentration over 1 mg g^{-1} P. In the current study, we found only small levels of fertilisation caused P concentrations to rise above values found in natural ecosystems, with peak concentrations, after P application approximately 2-4 times that found in native habitats.

Although initial seedling growth rates across species are often positively related to seed mass (e.g. Westoby et al. 1996), this was not the case over the 136 days of growth in our current study, possibly because seedling growth was no longer relying on stored seed reserves. Indeed, despite having a seed mass approximately one third of that of the largest seeded species, *Atriplex nummularia* achieved the greatest dry mass. In addition, *Atriplex nummularia* had the highest P-response efficiency values of our six study species, and a higher P-use efficiency than all 11 perennial herbaceous legumes that were investigated by Pang *et al*. (2010) as potential pasture legumes for use conditions of under low P availability.

While high P-use efficiency between soil P concentrations of 0 and 4 mg kg^{-1} might relate to seed P concentrations, P-use efficiency was also high between 4 – 8 and 8 – 15 mg P kg^{-1}. Thus, *Atriplex nummularia* appears to have an ability to acquire P and use it efficiently for biomass production even in low P environments. Consequently, this species has potential for rapid growth and establishment in the low P environments of post mining restored sites.

Three of the study species (the two *Acacia* species and *Eucalyptus torquata*) were able to maintain their internal root and shoot P-concentrations at a constant level at low, but not at high, external P concentrations. This is similar to the findings by de Campos *et al.* (2013) who reported the ability of two *Acacia* species (*Acacia truncata* and *Acacia xanthina*) to regulate internal P-concentrations at low external P-concentrations. However, at higher external P concentrations root and shoot P-concentrations increased in all six of our study species. Similar to our current study, de Campos *et al*. (2013) also reported a mixed result in terms of the effect of increasing P on biomass accumulation: *Acacia truncata* was unresponsive in terms of biomass to high P, whilst *Acacia xanthina* exhibited declining biomass as P increased. These results suggest that the growth response to elevated P, even within co-occurring members of a genus, is unpredictable as also reported for the genus *Banksia* (de Campos *et al*. 2013).

Our data suggest that even low concentrations of soil P can have negative effects on plant growth for a range of woody species from the GWW. Based on a soil dry bulk density of 1 g cm^{-3} and applied P being limited to the top 100 mm of soil, the concentrations at which either negative effects were observed, or growth showed no further response (15 or 30 mg P kg^{-1} soil dependent on species), are equivalent to field application rates of 15 or 30 kg P ha^{-1}. These application rates are at the low end of those used in many mine site restoration programmes: 25-80 kg P ha^{-1} are commonly applied (e.g. Holmes 2001; Spain et al. 2015; Standish *et al*. 2015; KCGM 2015). Consequently, the rates of P applied to mine restoration have the potential to reduce, rather than increase, growth of some species. While fungal associations can moderate some of the negative effects of applied-P for some species (Kariman *et al*. 2014), the application of P has the potential to change the outcome of competitive interactions by differentially affecting growth of individual species.

Applied P can also result in an increased abundance of weeds in restored sites (Holmes 2001; Daws *et al*. 2013, 2015). For example, in five-year-old restored jarrah forest in Western Australia weed density increased from close to zero to more than 5 stems m^{-2} as the P-application rate increased from 0 to 80 kg ha^{-1} (Daws et al. 2015). Negative effects of applied-P on plant growth have also been reported in previous studies of mine restoration where applied-P increased mortality or reduced abundance for longer-lived, slow growing species (e.g. Holmes 2001; Daws *et al*. 2013, 2015). However, in these field-based studies it was not possible to determine if the reduced performance of native species resulted from increased competition with weed species, direct negative effects of applied-P on growth, or both. Nonetheless, our current results support the hypothesis that fertiliser application may negatively affect the competitiveness of some species by actually reducing their growth rates. When combined with applied-P increasing the growth and competitiveness of other species (e.g. weeds and annuals), this introduces an important consideration for the use of applied-P in the restoration of natural plant communities.

Conclusions

For six plant species from the GWW we found that while low levels of applied-P increased plant growth, above an application rate of 15 mg kg^{-1} effects on plant growth became unpredictable: growth either declined or showed no further response to increasing external P concentrations. Overall our data suggest that careful consideration should be given to designing appropriate fertiliser regimes for restored sites to avoid the risk that fertiliser addition has the unwanted outcome of decreasing growth and survival of the target native species whilst increasing the abundance of weeds.

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Conflicts of Interest

The authors declare no conflict of interest.

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Table 1. Details of the six study species used in the pot-based experiment of the effect of applied-P on plant growth.

Species	**Genus**	**Seed mass (mg)**	**Growth form**
Acacia acuminata Benth.	Fabaceae	15.2	Shrub or tree
Acacia hemiteles Benth.	Fabaceae	22.1	Shrub
Atriplex nummularia Lindl.	Chenopodiaceae	6.86	Shrub
Atriplex vesicaria Heward ex Benth.	Chenopodiaceae	5.77	Shrub
Eucalyptus torquata Luehm.	Myrtaceae	5.18	Tree
Maireana triptera (Benth.) Paul G. Wilson	Chenopodiaceae	12.54	Shrub

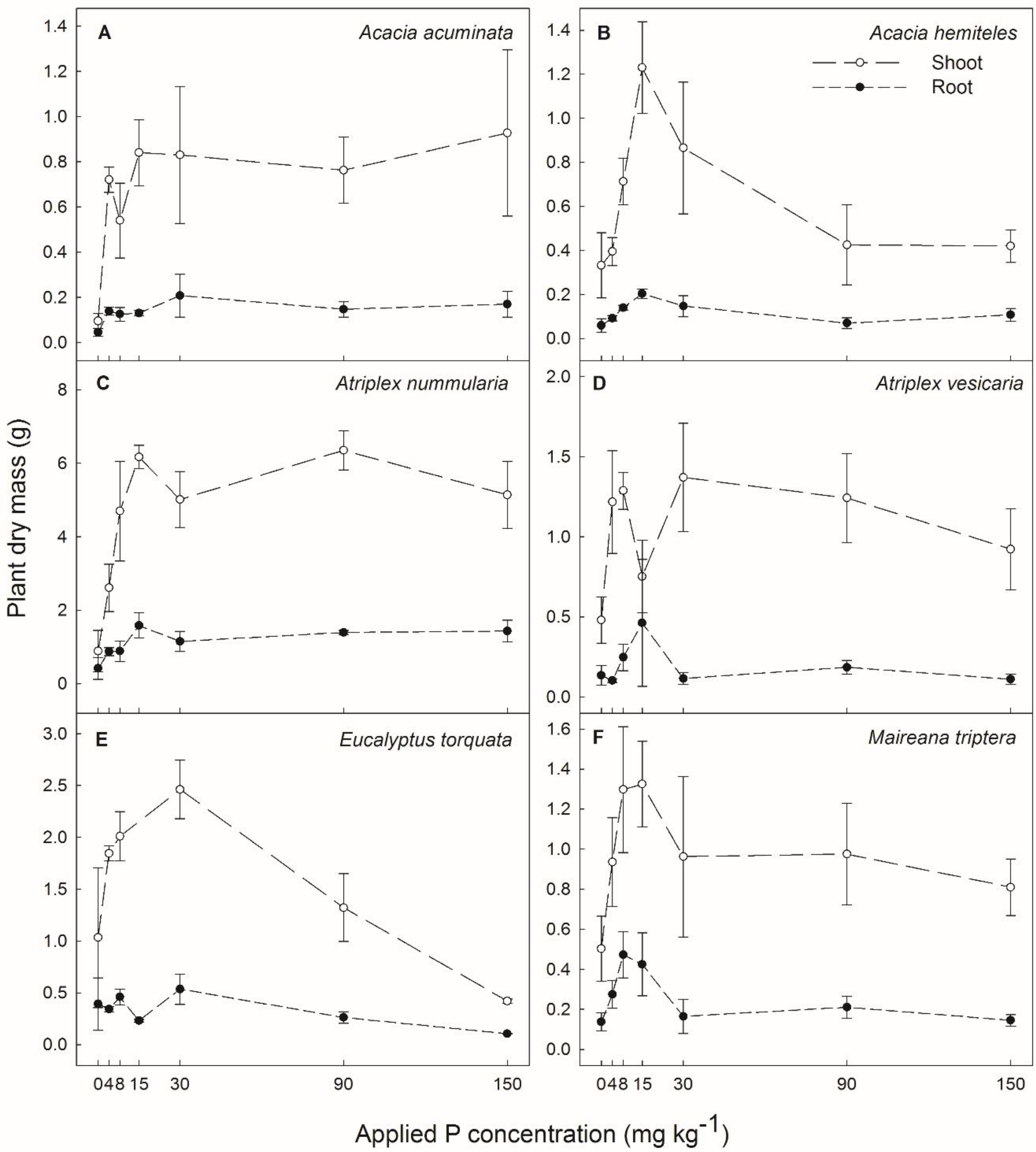
Figure legends:

Fig. 1. The effect of a range of external P concentrations on dry mass of six woody species from the Great Western Woodlands grown for 136 days in washed river sand at a range of external soil P concentrations, n=4 +/- standard error of the mean.

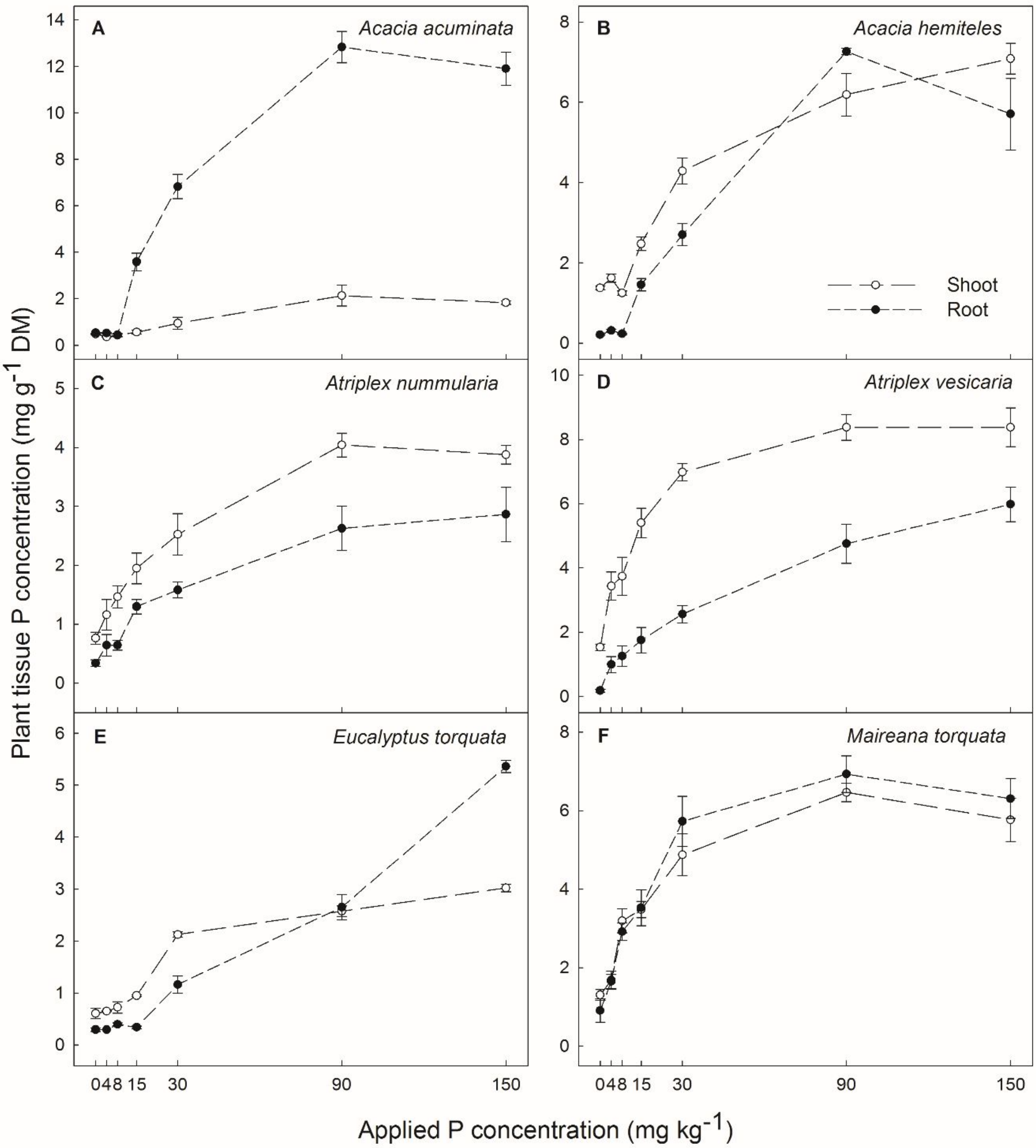
Fig.2. Comparison of root and shoot P concentrations for six woody species from the Great Western Woodlands grown for 136 days in washed river sand at a range of external P concentrations, n=4 +/- standard error of the mean. Dotted lines parallel to the x-axis represent phosphorus concentrations in shoot tissue for the plant families as reported in the natural flora of south west Western Australia (Foulds 1993).

Fig.3. Phosphorus-response efficiency (yield response per unit of applied P) measured for six P-application ranges (0-4, 4-8, 8-15, 15-30, 30-90 and 90-150 mg P kg^{-1} sand) for six woody species from the Great Western Woodland, n=4 +/- standard error of the mean.

Figure 1.

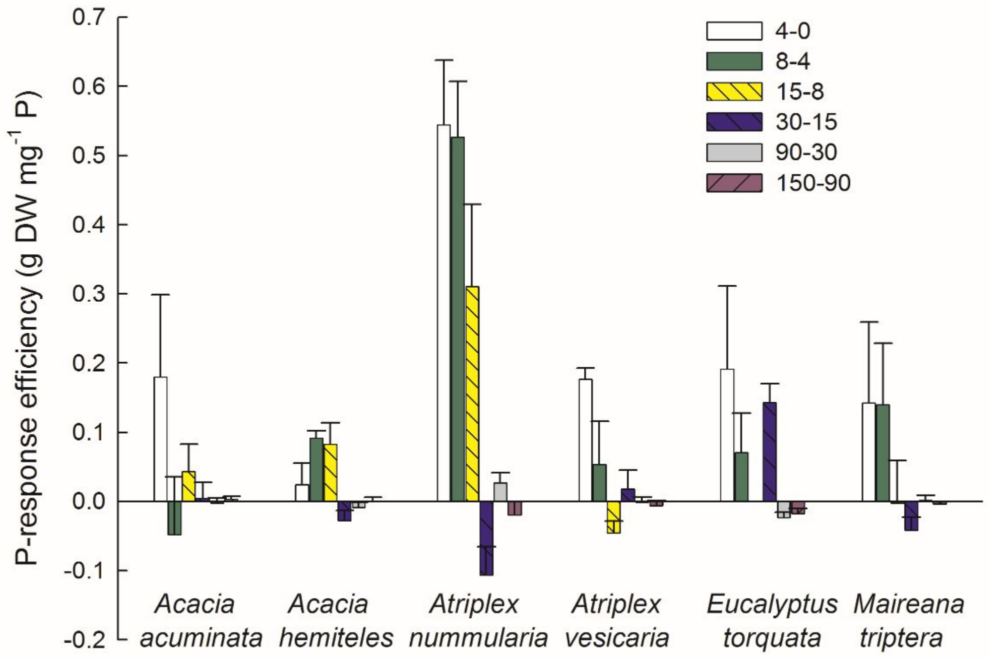

A
Acacia acuminata
B
Acacia hemiteles
Shoot
Root
C
Atriplex nummularia
D
Atriplex vesicaria
E
Eucalyptus torquata
F
Maireana triptera
Plant dry mass (g)
Applied P concentration (mg kg^{-1})
0 4 8 15 30 90 150

1 **Figure 2.**


A
Acacia acuminata
B
Acacia hemiteles
C
Atriplex nummularia
D
Atriplex vesicaria
E
Eucalyptus torquata
F
Maireana torquata
Shoot
Root
Plant tissue P concentration (mg g-1 DM)
Applied P concentration (mg kg-1)
0 4 8 15 30 90 150

2

Figure 3.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
P-response efficiency (g DW mg^{-1} P)
4-0
8-4
15-8
30-15
90-30
150-90
Acacia acuminata
Acacia hemiteles
Atriplex nummularia
Atriplex vesicaria
Eucalyptus torquata
Maireana triptera

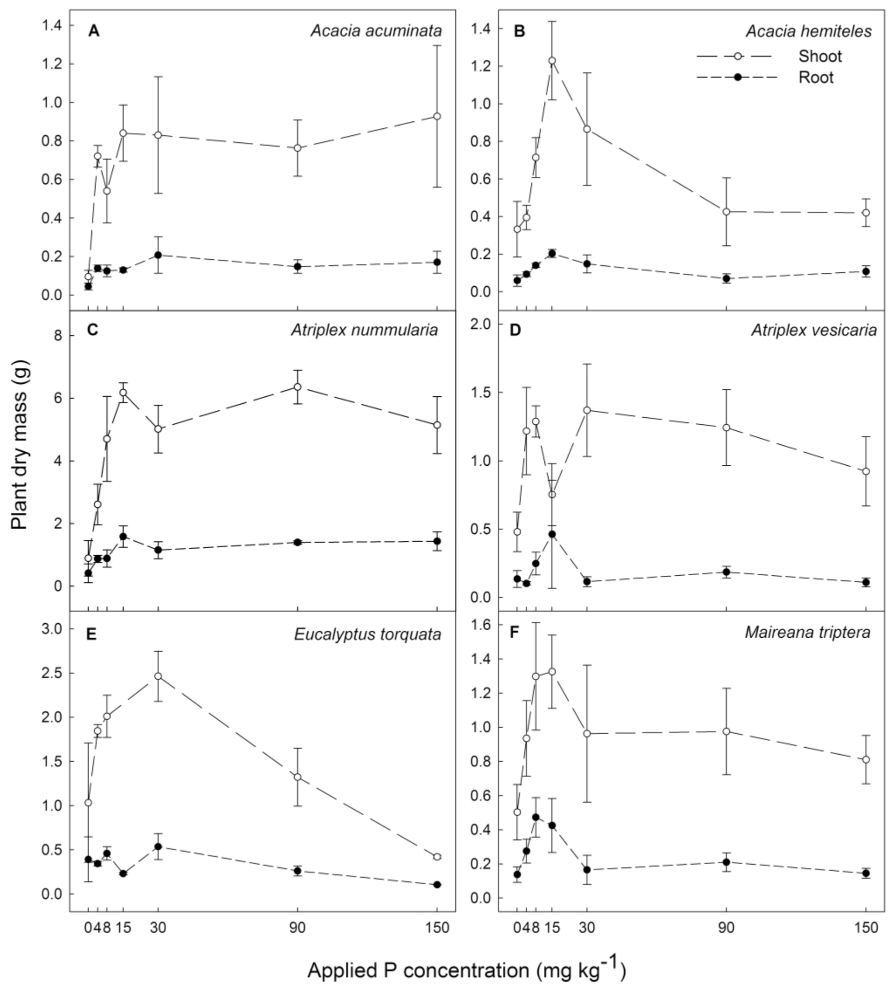


Fig. 1. The effect of a range of external P concentrations on dry mass of six woody species from the Great Western Woodlands grown for 136 days in washed river sand at a range of external soil P concentrations, n=4 +/- standard error of the mean.

863x1117mm (150 x 150 DPI)

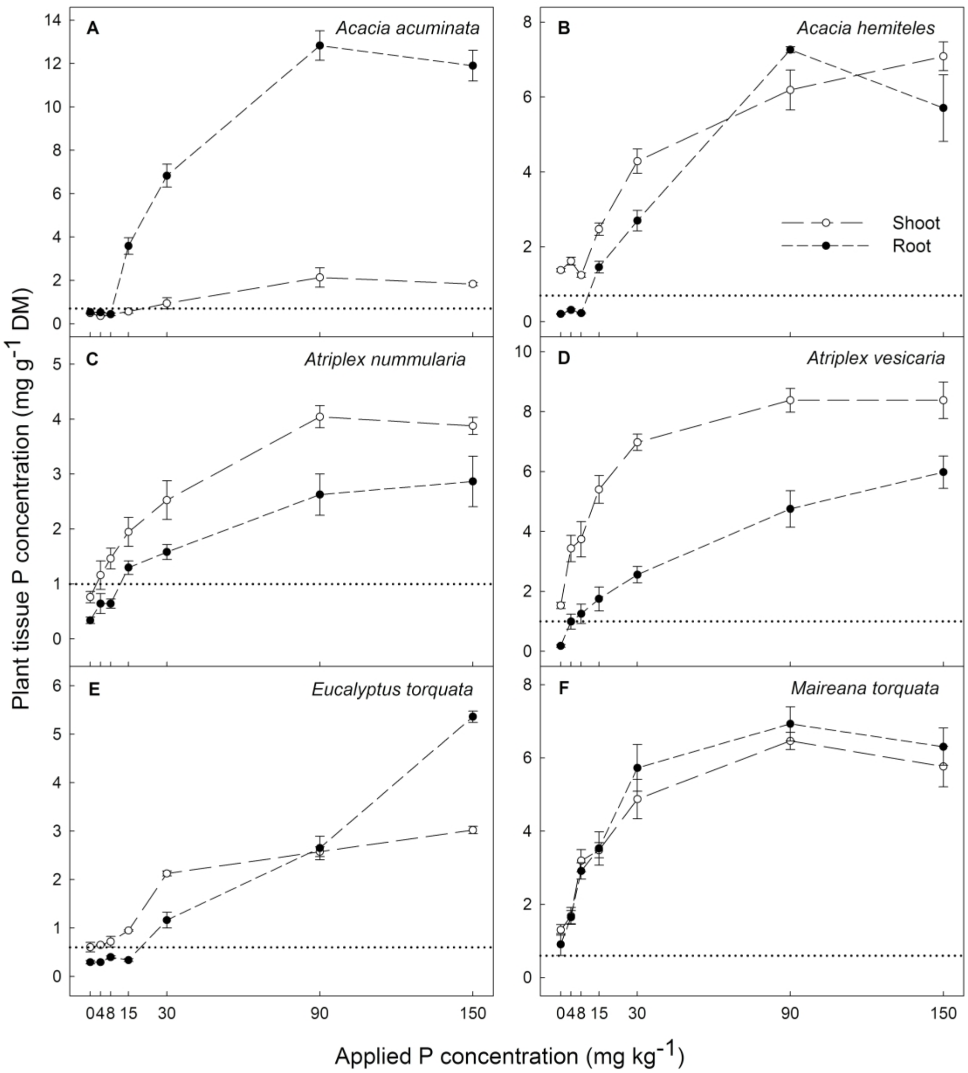


Fig.2. Comparison of root and shoot P concentrations for six woody species from the Great Western Woodlands grown for 136 days in washed river sand at a range of external P concentrations, n=4 +/- standard error of the mean. Dotted lines parallel to the x-axis represent phosphorus concentrations in shoot tissue for the plant families as reported in the natural flora of south west Western Australia (Foulds 1993).

849x956mm (150 x 150 DPI)

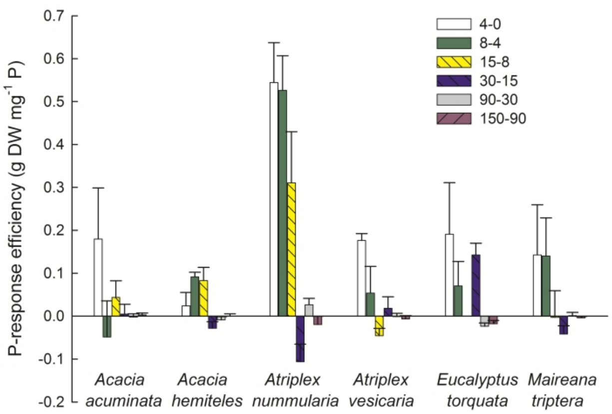


Fig.3. Phosphorus-response efficiency (yield response per unit of applied P) measured for six P-application ranges (0-4, 4-8, 8-15, 15-30, 30-90 and 90-150 mg P kg-1 sand) for six woody species from the Great Western Woodland, n=4 +/- standard error of the mean.

846x1095mm (150 x 150 DPI)

Species	Genus	Seed mass (mg)	Seed P concentration (mg g^{-1} DM)	Growth form
Acacia acuminata Benth.	Fabaceae	15.2	2.1	Shrub or tree
Acacia hemiteles Benth.	Fabaceae	22.1	1.9	Shrub
Atriplex nummularia Lindl.	Chenopodiaceae	6.86	0.7	Shrub
Atriplex vesicaria Heward ex Benth.	Chenopodiaceae	5.77	0.7	Shrub
Eucalyptus torquata Luehm.	Myrtaceae	5.18	0.6	Tree
Maireana triptera (Benth.) Paul G. Wilson	Chenopodiaceae	12.54	0.7	Shrub

112x61mm (150 x 150 DPI)